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American Journal of Epidemiology

Vol. 159, No. 9
Printed in U.S.A.
DOI: 10.1093/aje/kwh123

Reproducibility, Validity, and Responsiveness to Change of a Short Questionnaire for Measuring Fruit and Vegetable Intake

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Received for publication May 9, 2003; accepted for publication December 4, 2003.

The reproducibility, relative validity, and responsiveness to change of an eight-item food frequency questionnaire designed to measure fruit and vegetable consumption was assessed among 157 women (mean age = 41 years) in the Netherlands from spring 2001 to spring 2002. Plasma concentrations of vitamin C and total and specific carotenoids served as biomarkers against which validity was assessed. The questionnaire was completed and biomarker concentrations were determined three times: immediately preceding and following a controlled intervention of 1 month aimed at increasing fruit and vegetable consumption and 1 year after the start of the intervention. The 1-month and 1-year reproducibility of total fruit and vegetable consumption assessed in the control group was 0.80 and 0.79 (Spearman's *r*). Correlations between consumption and plasma carotenoids and vitamin C at baseline were 0.39 and 0.37, respectively, for fruits and 0.24 and 0.26, respectively, for vegetables. Correlations between changes in consumption and plasma carotenoids and vitamin C were 0.32 and 0.33, respectively, for fruits and 0.28 and 0.30, respectively, for vegetables. On the basis of similar correlations reported in the literature, the authors conclude that the questionnaire appears to be suitable for ranking individuals according to their consumption of fruits and vegetables and according to changes in their consumption. However, the validity of the questionnaire remains to be established in males, other age groups, and populations of lower educational levels.

ascorbic acid; carotenoids; fruit; nutrition assessment; questionnaires; reproducibility of results; validation studies [publication type]; vegetables

Abbreviations: FFQ, food frequency questionnaire; SD, standard deviation.

Many observational studies in humans suggest that consumption of fruits and vegetables is beneficial for the prevention of cancer (1–4) and cardiovascular disease (5–8). On the basis of these findings, public health authorities in several countries have defined recommended intake levels of these foods. Dutch authorities have recommended intake levels of 200 g of vegetables and two pieces (approximately 250 g) of fruit per day (9, 10). Similar amounts are recommended in other Western countries (11). Since many consumers do not meet these recommendations (12), fruit and vegetable promotion programs have been launched in order to increase consumption of these foods (13).

Evaluating the effectiveness of these health promotion interventions requires a measuring instrument that is able to determine changes in fruit and vegetable consumption (14). Such an instrument must be short and simple in order to minimize participants' time and effort and thereby maximize participation rates, and to minimize the intervention effect that might emanate from using intensive research methods for assessing dietary intake. For this reason, we decided to develop and validate a concise food frequency questionnaire

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(FFQ), a self-administered instrument for estimating habitual intake of fruits and vegetables.

Validation of FFQs can be done in various ways; comparisons with diet records or recalls have most frequently been reported (15). Another method is to compare the results of an FFQ with biochemical markers of dietary intake. The advantage of using biomarkers is that they provide an objective measure of intake whose measurement errors are essentially independent of the errors associated with FFQs (i.e., systematic overestimation or underestimation of consumption frequency or portion size of certain foods) (16). Therefore, correlations between fruit and vegetable consumption determined with a dietary assessment method, such as an FFQ, and biomarkers are not artificially inflated as a result of correlated errors in both methods, which can be the case when two similar methods are compared. Hence, in the present study, we used biomarkers as a method of validation instead of another method based on self-report, such as 24-hour recalls. Frequently used biomarkers for fruit and vegetable intake are levels of carotenoids and vitamin C in blood. A number of studies have shown that blood levels of carotenoids (17–21) and vitamin C (19, 21) are correlated with fruit and vegetable intake. Moreover, these biomarkers have been shown to be responsive to changes in fruit and vegetable consumption (22–27). However, correlations between fruit and vegetable intake and concentrations of the above biomarkers are modest, because the biomarkers are also influenced by physiologic factors such as absorption and metabolism.

Short (fewer than 15 items) FFQs designed to assess fruit and vegetable consumption have been used and validated in previous studies (28–34). However, all of these studies had a cross-sectional design, and only one (33) used biomarkers as an independent reference method. Our study, in contrast, determined fruit and vegetable consumption and plasma concentrations of vitamin C and carotenoids before and after an intervention aimed at increasing fruit and vegetable consumption. This was done among mothers of primary school-age children, a large population group in the Netherlands. We wanted to validate the FFQ for its ability to rank people according to their usual fruit and vegetable intake and according to changes in their fruit and vegetable intake—that is, to explore relations between FFQ estimates and biomarker estimates both cross-sectionally and longitudinally.

MATERIALS AND METHODS

Study population

From the population registry of the municipality of Maastricht, the Netherlands, we obtained a random sample containing 2,000 addresses of mothers with children aged 7–10 years. A letter was sent inviting the mother and the selected child in each family to participate. An additional 1,100 letters were distributed via elementary schools in Maastricht. The women had to be apparently healthy nonsmokers who agreed not to use vitamin supplements from 1 month before the first blood collection to the end of the study period. The letter explained that the study's purposes were to develop questionnaires assessing fruit and vegetable intake and to assess determinants of fruit and vegetable consumption (the latter in another part of the project not described here). A total of 207 volunteers were recruited (a 6.7 percent response rate). Data presented in this report concern only the mothers.

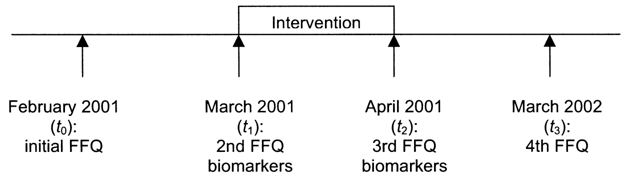

FIGURE 1. Design of a study evaluating the reproducibility, validity, and responsiveness of a food frequency questionnaire (FFQ) for assessing fruit and vegetable consumption, Maastricht, the Netherlands, 2001–2002.

Study design

Approval for the present study was obtained from the medical ethics committee of Maastricht University. The study design is shown in figure 1. In February 2001 (t_0), an initial FFQ accompanied by some questions on background characteristics (including age, marital status, and educational level) was sent to the participants, who returned it by mail. After 1 month (t_1), this FFQ was sent out again, and the participants were asked to complete the FFQ on the day or evening before they came to the study center to undergo blood sampling for determination of biomarkers. Participants handed in the questionnaire, and a venous blood sample was drawn during the morning. Subsequently, after prestratification of participants on fruit and vegetable intake at t_0 (above or below the median) to minimize initial differences in fruit and vegetable intake, participants were randomly allocated to either a 1-month dietary intervention aimed at increasing their fruit and vegetable consumption or no intervention (the control group). All measurements were repeated immediately after the intervention (t_2), as well as 1 year after the baseline measurements (t_3). Body mass index was calculated as weight (kg)/height (m)^2, determined at the study center.

FFQ

The FFQ (figure 2) was based on the FFQ originally described by Van Assema et al. (35). It had a reference period of 1 month and included the types or categories of fruits and vegetables consumed most frequently in the Netherlands (36). Potatoes were not explicitly included in the category "cooked vegetables," because potatoes are considered a staple food (not a vegetable) in the Netherlands. Participants were asked to indicate their usual portion size for each category of fruits and vegetables in open-ended questions on number of serving spoons (one serving spoonful = 50–60 g), pieces, or glasses (see figure 2). However, the FFQ in figure 2 is shown with closed-ended

The questions in this questionnaire refer to the **past month**. Please could you mark:

- **how often** you ate each product on average during the past month;
- **how much** of a product you took on average on a day when you ate or drank it.

Example:
Given that you ate bananas on two days a week during the past month, and you took one banana on such a day, you fill in:

How often **did you eat on average during the past month:**	**never** or less than 1 day a *month*	**1-3 days a** *month*	**1 day a week**	**2 days a week**	**3 days a week**	**4 days a week**	**5 days a week**	**6 days a week**	**7 days a week**	**On a day when you ate or drank this, *how much* did you take?** 1	2	3	4	5 or more	
Bananas	☐	☐	☐	☒	☐	☐	☐	☐	☐	☒	☐	☐	☐	☐	pieces

- If you did not take a product during the past month, please do not forget to mark the category "never or less than 1 day a month". In that case it is not necessary to report a portion size.
- In all other cases, please also mark the amount you eat or drink. Report the amount you **yourself** eat or drink, and not the amount for the whole family.
- Please read carefully every question and choose the answer that suits you best.

Now the questionnaire begins:

How often **did you eat on average during the past month:**	**never** or less than 1 day a *month*	**1-3 days a** *month*	**1 day a week**	**2 days a week**	**3 days a week**	**4 days a week**	**5 days a week**	**6 days a week**	**7 days a week**	**On a day when you ate or drank this, *how much* did you take?** 1	2	3	4	5 or more	
Vegetables, cooked (boiled, fried, steamed or cooked otherwise) *(also keep in mind vegetables in e.g., sauce)*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	serving spoons *(= 50 g)*
Raw vegetables and salad (lettuce, cucumber, tomato etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	serving spoons
Fruit and vegetable juice (e.g., orange juice, fresh or from a carton, tomato juice)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	glasses
Tangerines	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	pieces
Oranges, grapefruits, lemons or other citrus fruits *(no juice)*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	pieces
Apples and pears (excluding apple sauce)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	pieces
Bananas	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	pieces
Other fruits (also canned or jarred)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	pieces *(a bowl of e.g., berries also counts as one piece)*

FIGURE 2. The short food frequency questionnaire for assessing fruit and vegetable consumption that was used in the validation study, Maastricht, the Netherlands, 2001–2002.

questions for portion size, which makes it possible to read the questionnaire with an optical scanner. We were able to compare portion sizes of cooked vegetables, bananas, and tangerines when either open-ended questions or categories were used, and we found that mean portion sizes did not differ significantly between the two methods (mean portion sizes in servings for categories and open-ended questions, respectively, were 3.0 (standard deviation (SD), 0.8) and 3.0

(SD, 1.0) for cooked vegetables, 0.8 (SD, 0.4) and 0.8 (SD, 0.4) for bananas, and 0.3 (SD, 0.4) and 0.4 (SD, 0.5) for tangerines). Spearman's rank correlation coefficients for correlations between the portion sizes calculated with either method were 0.72 (cooked vegetables), 0.78 (bananas), and 0.80 (tangerines).

Population mean values were substituted for missing values on portion size for any particular item (1.3 percent of all items at t_1, t_2, and t_3). Consumption, in servings per day, was calculated by multiplying consumption frequency and portion size. One piece of fruit, one glass of juice, or one serving spoonful of vegetables was considered one serving. Population mean values for each item were used to replace missing values for fruit and vegetable intake in servings per day (0.3 percent of all items at t_1, t_2, and t_3). Consumption of the various items was summed to obtain total fruit consumption, total vegetable consumption, and fruit and vegetable consumption. Citrus fruit consumption was calculated as consumption of tangerines and other citrus fruit. Consumption of fruit and vegetable juice was considered to comprise mainly fruit juice (37) and thus was included in total fruit consumption and fruit and vegetable consumption.

Dietary intervention

The aim of the intervention was to accomplish an increase in fruit and vegetable consumption that could be achieved through an intensive nutrition promotion campaign. Participants in the dietary intervention group were asked to consume at least 200 g of fresh vegetables and two pieces of fruit per day (the Dutch recommended intake level for fruits and vegetables) over a period of 1 month. To increase compliance, packets containing sufficient amounts of fruits and vegetables to feed the entire family the recommended amounts were delivered weekly to the participants' homes free of charge, together with a regular newsletter and recipes. Women in the control group received neither fruit and vegetable packets nor further information on fruits and vegetables.

Analysis of biomarkers

The participants were requested to come to the study center after fasting overnight. Venous blood samples were drawn between 7:00 a.m. and 11:00 a.m. in two K_2 ethylenediaminetetraacetic acid-coated plastic tubes (Becton Dickinson Vacutainer Systems, Plymouth, United Kingdom) and were immediately placed on ice in the dark. Total ascorbic acid concentration was determined by high performance liquid chromatography with fluorometric detection according to the method developed by Speek et al. (38). Plasma levels of carotenoids (α-carotene, β-carotene, lutein, β-cryptoxanthin, and lycopene) were analyzed with high performance liquid chromatography with ultraviolet detection according to the method of Hess et al. (39), with modifications described by Oostenbrug et al. (40). A more detailed description is given elsewhere (41). Although a few respondents (two at t_1, seven at t_2, and seven at t_3) were not in the fasting state, exclusion of these persons from the analysis did not substantially affect the results.

Data analysis

Respondents were excluded from further analysis if their FFQs contained more than 20 percent missing values. The data collected at t_3 to determine reproducibility were only analyzed for control subjects who had less than 20 percent missing values on the FFQ completed at t_3 and who had also been included in the analyses at t_1 and t_2. Because of the skewed distribution of the dietary intake data, nonparametric statistics were used in the cross-sectional and longitudinal validation analyses. The 1-month and 1-year reproducibility of food items was calculated as Spearman's rank correlation coefficients in control subjects. We calculated agreement in absolute intake by classifying participants according to their fruit and vegetable consumption in three categories and calculating the percentage agreement in classification between the three measurements. Cutoff points for the categories were 1.5 and 2.5 servings per day for fruits and 2.5 and 4.0 servings per day for vegetables. These points were chosen to create groups of participants consuming approximately the recommended amounts of fruits and vegetables and participants consuming clearly less than or more than this amount.

The effect of the intervention on fruit and vegetable consumption and biomarkers was calculated as the mean change in the intervention group minus the mean change in the control group. The statistical significance of the intervention effects was tested with the Mann-Whitney *U* test.

As a measure of validity, Spearman's correlation coefficients for correlations between (changes in) dietary intake assessed with the FFQ and (changes in) plasma carotenoids and vitamin C were used. Cross-sectional correlation coefficients were corrected for body mass index. In order to include both participants with clear-cut changes and those with low change or no change in fruit and vegetable consumption, participants from the control group were also included in the longitudinal validation analyses. We verified that intervention and control participants were not essentially different in terms of their plasma response to changes in fruit and vegetable consumption (data not shown). Correlation coefficients were calculated for correlations between biomarkers and total fruit, total vegetable, total fruit and vegetable, and citrus fruit consumption. The latter, more specific correlations were calculated because citrus fruits are known to have an especially high content of vitamin C and β-cryptoxanthin.

Educational level was classified as low (primary school), middle (high school), or high (polytechnic academy or university). Differences at baseline between the intervention and control groups were tested using the chi-squared test for homogeneity (marital status and educational level) or the independent-samples *t* test (age and body mass index).

RESULTS

Participants

Of the 207 women who agreed to participate, 194 started the study and 191 completed the initial questionnaire (t_0; figure 1). The second questionnaire just preceding the intervention was completed by, and a blood sample was obtained

TABLE 1. Fruit and vegetable intakes of healthy women in March 2001 (t_1), April 2001 (t_2), and March 2002 (t_3) and reproducibility (Spearman's *r*) of a short food frequency questionnaire in the control group, Maastricht, the Netherlands

	Mean intake (servings/day)			Spearman correlation	
	t_1 (baseline) (n = 73)	t_2 (1 month) (n = 73)	t_3 (1 year) (n = 60)	t_1–t_2	t_1–t_3
Total vegetables	3.5 (1.5)†	3.4 (1.4)	3.4** (1.3)	0.73	0.81
Cooked vegetables	2.3 (1.0)	2.2 (0.9)	2.3* (0.9)	0.66	0.70
Raw vegetables	1.2 (1.0)	1.2 (0.8)	1.1 (0.9)	0.78	0.77
Total fruits‡	1.8 (1.0)	1.7* (0.9)	1.7 (1.0)	0.80	0.62
Citrus fruits§	0.4 (0.4)	0.3* (0.3)	0.4 (0.4)	0.81	0.58
Apples/pears	0.5 (0.5)	0.6 (0.5)	0.4* (0.5)	0.79	0.54
Bananas	0.2 (0.2)	0.1 (0.2)	0.1 (0.1)	0.67	0.64
Other fruits	0.2 (0.3)	0.2 (0.3)	0.2 (0.6)	0.49	0.31
Fruit and vegetable juices	0.6 (0.7)	0.5 (0.6)	0.5 (0.6)	0.82	0.62
Total fruits and vegetables‡	5.4 (2.1)	5.1* (2.0)	5.1** (1.9)	0.80	0.79

* $p < 0.05$; ** $p < 0.01$ (significantly different from consumption at t_1 (two-sided p value; Wilcoxon signed rank test; comparison between t_1 and t_3 was based on 60 participants)).

† Numbers in parentheses, standard deviation.

‡ Including fruit juice and vegetable juice.

§ Excluding juice.

from, 174 women (86 participants randomized to the control group and 88 randomized to the intervention group). Immediately after the intervention period (t_2; figure 1), questionnaires and blood sampling were completed by 165 participants (77 controls and 88 intervention subjects). Eight respondents were excluded from further analysis because their FFQs contained more than 20 percent missing values (two intervention subjects at t_1 and two intervention subjects and four control subjects at t_2), leaving a total number of 157 participants. One participant did not provide enough blood for the analysis of vitamin C at t_1 and was excluded from the validation analyses with vitamin C. The mean age of the 157 women was 41 years (range, 29–50), and the mean body mass index was 24.0 (range, 18.7–35.9). Ninety-two percent of the women were married or living with a partner; 45 percent had a high educational level, 50 percent had an intermediate level, and 5 percent had a low level. As expected in a randomized design, the intervention and control groups were similar with regard to those variables (data not shown). At 1 year of follow-up (t_3; figure 1), 149 participants (68 controls and 81 intervention subjects) completed the FFQ and gave blood. Sixty control subjects had less than 20 percent missing values on the FFQ completed at t_3 and had also been included in the analyses at t_1 and t_2. Reasons for dropping out (after returning the initial questionnaire) were a lack of time or interest (n = 27), illness at the time of blood sampling (n = 6), practical reasons (for example, it was not possible to make a suitable appointment for blood collection) (n = 7), and personal circumstances or unknown reasons (n = 5). Compared with the participants who completed the study, the ones who dropped out were significantly younger (39 years vs. 41 years) and consumed significantly fewer vegetables (2.9 servings per day vs. 3.5 servings per day assessed with the initial FFQ). The 26 controls at t_1 who did not complete the rest of the study consumed significantly fewer vegetables than the 60 controls who were full participants (3.0 servings per day vs. 3.8 servings per day assessed with the initial FFQ).

Reproducibility

Table 1 shows the fruit and vegetable consumption of the controls at t_1, t_2 and t_3, as well as the reproducibility of the FFQ after 1 month and 1 year. Fruit and vegetable consumption at t_2 differed from consumption at t_1 for citrus fruits, total fruits, and total fruits and vegetables. Consumption at t_3 was significantly lower than consumption at t_1 for total vegetables, cooked vegetables, apples/pears, and total fruits and vegetables. Correlation coefficients for correlations between consumption levels measured at t_1 and t_2 were 0.66–0.82, with the exception of "other fruits" (r = 0.49). Compared with the 1-month reproducibility, the 1-year reproducibility was similar for vegetable consumption but lower for fruit consumption.

Table 2 illustrates the reproducibility of the FFQ in terms of absolute agreement between the three measurements. The questionnaire classified 57–74 percent of the participants in the same category of fruit and vegetable consumption when the FFQ was administered repeatedly. Of the persons who were classified in a different category, most had moved down one category.

Intervention effects

Table 3 shows the changes in fruit and vegetable consumption and biomarker concentrations in the intervention group after 1 month of dietary intervention. Mean plasma concentrations in the control group at baseline did not significantly

TABLE 2. Agreement between repeated administrations* of a short food frequency questionnaire after classification of healthy women from the control group† into three categories‡ according to their fruit and vegetable consumption, Maastricht, the Netherlands, 2001–2002

	% of participants§				
	Same class	One class up	One class down	Two classes up	Two classes down
Vegetables					
t_1–t_2	59	18	23	0	0
t_1–t_3	70	7	23	0	0
Fruits					
t_1–t_2	74	6	18	3	0
t_1–t_3	57	12	28	0	2

* t_1, March 2001; t_2, April 2001; t_3, March 2002.

† n = 73 at t_1 and t_2; n = 60 at t_3.

‡ Class boundaries: for fruits, 1.5 and 2.5 servings per day; for vegetables, 2.5 and 4.0 servings per day. The low, intermediate, and high categories at t_1 included 37%, 41%, and 22% of the participants for fruit consumption and 26%, 41%, and 33% of the participants for vegetable consumption.

§ Percentages may not add up to 100% because of rounding error.

differ from those in the intervention group. The intervention effect was 1.3 servings per day for vegetables and 1.1 servings per day for fruits. The strongest effects in the categories of fruits and vegetables were observed for citrus fruits and cooked vegetables (data not shown), respectively. Mean plasma vitamin C and carotenoid concentrations also increased significantly as a result of the intervention. The carotenoid that increased most in concentration was β-cryptoxanthin.

Relative validity

Table 4 presents correlations of plasma carotenoid and vitamin C concentrations with fruit and vegetable consumption assessed with the FFQ in the total study population. Although many correlation coefficients reached statistical significance, the degree of correlation was modest (0–0.57). Plasma lycopene concentrations were not correlated with any of the fruit and vegetable categories.

Table 5 shows that in the whole study population, changes in fruit and vegetable consumption were modestly correlated with changes in plasma concentrations of vitamin C and all carotenoids except lycopene. The highest correlation was between changes in citrus fruit consumption and changes in β-cryptoxanthin (r = 0.46).

DISCUSSION

In the present study, we determined the reproducibility and relative validity of a short FFQ designed to measure (changes in) fruit and vegetable intake. After 1 month, the

TABLE 3. Mean fruit and vegetable intakes and plasma vitamin C and carotenoid concentrations in 84 healthy women at baseline (t_1; March 2001) and after 1 month of nutrition intervention (t_2; April 2001) aimed at increasing fruit and vegetable intake, Maastricht, the Netherlands

	Mean intake or plasma concentration		Mean intervention effect†
	t_1 (baseline)	t_2 (1 month)	
Dietary intake (servings/day)			
Total vegetables	3.4 (1.7)‡	4.6 (1.4)	1.3* (0.2)
Total fruits§	1.9 (1.1)	2.9 (1.2)	1.1* (0.1)
Citrus fruits¶	0.4 (0.5)	0.7 (0.6)	0.4* (0.1)
Total fruits and vegetables§	5.3 (2.3)	7.5 (2.0)	2.4* (0.3)
Plasma concentration (μmol/liter)			
Vitamin C#	55.07 (13.90)	61.54 (10.68)	10.05* (1.73)
Total carotenoids	2.04 (0.81)	2.28 (0.91)	0.32* (0.07)
α-carotene	0.18 (0.20)	0.19 (0.19)	0.03* (0.01)
β-carotene	0.58 (0.48)	0.64 (0.44)	0.07* (0.03)
Lutein	0.39 (0.14)	0.48 (0.18)	0.09* (0.02)
β-cryptoxanthin	0.62 (0.33)	0.70 (0.31)	0.14* (0.03)
Lycopene	0.28 (0.11)	0.26 (0.11)	0.01 (0.02)

* $p < 0.01$ (intervention effect differed significantly from zero; two-sided p value; Mann-Whitney U test).

† Change in intervention group minus change in control group.

‡ Numbers in parentheses, standard deviation.

§ Including fruit juice and vegetable juice.

¶ Excluding juice.

\# n = 83.

TABLE 4. Spearman's correlation coefficients† for correlations between fruit and vegetable intake and plasma concentrations of vitamin C and carotenoids determined in March 2001 (t_1) in 157 healthy women, Maastricht, the Netherlands

	Vitamin C‡	Total carotenoids	α-carotene	β-carotene	Lutein	β-cryptoxanthin	Lycopene
Total vegetables	0.26**	0.24**	0.37**	0.17*	0.26**	0.16	0.04
Total fruits§	0.37**	0.39**	0.23**	0.23**	0.13	0.42**	0
Citrus fruits#	0.32**	0.27**	0.06	0.04	0.03	0.57**	–0.07
Total fruits and vegetables§	0.37**	0.37**	0.36**	0.23**	0.23**	0.31**	0.02

* $p < 0.05$; ** $p < 0.01$ (two-sided p value).
† Adjusted for body mass index.
‡ $n = 156$.
§ Including fruit juice and vegetable juice.
# Excluding juice.

reproducibility of the questionnaire was 0.73 for total vegetable consumption and 0.80 for total fruit consumption. After 1 year, the reproducibility of vegetable consumption was similar (r = 0.81), whereas the reproducibility of fruit consumption was lower than it was after 1 month (r = 0.62). With regard to the relative validity of the FFQ, positive modest correlations were found between fruit and vegetable consumption and biomarkers and between changes in both measures.

The reproducibility of an FFQ is influenced by properties of the FFQ itself, respondents' memory, and actual changes in food intake. Since mean fruit intake did not change from baseline to 1 year after baseline (table 1), it seems likely that individual shifts in fruit consumption would explain the lower reproducibility of fruit consumption 1 year after the first administration of the questionnaire. Alternatively, fruit consumption may be more subject to interindividual variation than vegetable consumption, or respondents may have more difficulty reporting their fruit consumption.

Regarding the validity of the FFQ, the use of biomarkers as a reference method for validating an FFQ as in the present study has both advantages and disadvantages. Measurement errors in FFQ estimates (e.g., overreporting of fruit and vegetable intake) are essentially independent of measurement errors in determination of plasma carotenoid and vitamin C concentrations (caused during collection, processing, and analysis of blood samples). This minimizes the problem of correlated errors in both methods, which could lead to artificially high correlation coefficients. However, a limitation of using plasma concentrations of carotenoids and vitamin C as biomarkers is that they are influenced not by nutrition alone but also by biologic factors such as absorption and metabolism; this causes correlations with fruit and vegetable consumption to be modest (42). Additionally, correlations between FFQ estimates and biomarkers could be confounded by other variables such as plasma cholesterol or body mass index. Adjustment for body mass index was done only in the cross-sectional analyses, because intraindividual changes in biomarkers, used in the longitudinal analyses, are unlikely to be influenced by nondietary factors. Adjusted correlation coefficients did not differ substantially from unadjusted coefficients (data not shown), indicating that body mass index was not an important confounder. We eliminated the effect of sex, smoking, and vitamin supplement use on biomarkers by including in our study only nonsmoking women who did not use supple-

TABLE 5. Spearman's correlation coefficients for correlations between changes in fruit and vegetable intake and changes in plasma concentrations of vitamin C and carotenoids determined between March 2001 (t_1) and April 2001 (t_2) in 157 healthy women, Maastricht, the Netherlands

	Vitamin C†	Total carotenoids	α-carotene	β-carotene	Lutein	β-cryptoxanthin	Lycopene
Total vegetables	0.30**	0.28**	0.29**	0.20*	0.28**	0.29**	–0.11
Total fruits‡	0.33**	0.32**	0.33**	0.24**	0.26**	0.37**	–0.06
Citrus fruits§	0.30**	0.32**	0.21**	0.13	0.26**	0.46**	–0.00
Total fruits and vegetables‡	0.37**	0.37**	0.36**	0.26**	0.34**	0.39**	–0.08

* $p < 0.05$; ** $p < 0.01$ (two-sided p value).
† $n = 156$.
‡ Including fruit juice and vegetable juice.
§ Excluding juice.

ments, but we did not control for blood lipid levels, since this has been shown not to substantially alter coefficients for correlation between fruit and vegetable intake and blood carotenoids (18–20, 43–45). A final limitation in the use of correlation coefficients as a measure of validity is that they can only be interpreted in terms of ranking individuals according to their fruit and vegetable intake, and because of the lack of a "gold standard," the validity of the FFQ in terms of absolute intake cannot be established. This is illustrated by the fact that a substantial number of participants fell into a different category of absolute intake when the FFQ was administered again after 1 month and 1 year.

Results from a number of previous studies in which FFQs or other instruments designed to measure fruit and vegetable intake were validated have been reported in the literature. An overview was given by Van Assema et al. (35) and Kim and Holowaty (46). To our knowledge, only one study (33) used biomarkers to validate three short FFQs. Therefore, we compared our results mostly with findings from more extensive questionnaires which were used to calculate correlations between fruit and vegetable intake and plasma or serum concentrations of carotenoids and/or vitamin C (17, 18, 21, 33, 47, 48). Two studies (18, 21) found higher correlations between fruit and vegetable consumption and biomarkers. The fact that in both of the above studies participants were selected on the basis of their high or low fruit and vegetable consumption may explain the relatively high correlations seen as compared with our study. Correlations similar to or lower than those in the present study were found in four studies (17, 33, 47, 48). Correlations between biomarkers and fruit and vegetable intake measured with dietary histories in which food consumption was recorded in terms of frequencies and quantities (19, 20) were similar to the correlations we observed.

We are aware of only one study that determined the ability of an FFQ to measure changes in food intake (49). This study showed low correlations between changes in intake over a 6-year period as measured with the FFQ and a dietary history method (r = 0.22 and r = 0.12 for fruits and vegetables, respectively). The few previous studies that related changes in fruit and vegetable consumption to changes in plasma or serum carotenoids or vitamin C all used diet records (23, 24, 26). Zino et al. (23) found correlations between change in number of servings of fruits and vegetables and change in plasma concentrations of α-carotene (r = 0.29), β-carotene (r = 0.09), and vitamin C (r = 0.25) after 4 weeks of dietary intervention. Maskarinec et al. (26) and Le Marchand et al. (24) found correlations of 0.52 and 0.69, respectively, between differences in total carotenoids and fruit and vegetable consumption after a 6-month or 3-month intervention period. The latter authors also calculated correlations between changes in total fruit and vegetable intake and plasma concentrations of specific carotenoids and vitamin C that were generally lower than the correlations we observed, except for lycopene. However, they found reasonably good correlations between some specific (groups of) vegetables and plasma concentrations of specific carotenoids. Differences between correlations found in the above studies and the present study presumably result from differences in intervention effect, study population, time frame, and dietary assessment method.

Some of the correlations between changes in fruit and vegetable intake and biomarker concentrations in the present study—for example, between changes in total vegetable consumption and plasma β-cryptoxanthin concentrations—were unexpected based on the carotenoid contents of the foods. This might have resulted from a concomitant increase in (citrus) fruit consumption with an increase in vegetable consumption after the intervention period.

We did not observe any associations between fruit and vegetable intake and plasma lycopene concentrations. This lack of association has been found in previous studies (18, 20, 33, 50). A possible explanation may be that major sources of lycopene (including tomatoes, ketchup, and pizza) were not asked about separately but were either grouped under "cooked vegetables" and "raw vegetables/salad" or not included in the FFQ. Since tomato consumption assessed with a more elaborate FFQ was not correlated with plasma lycopene concentrations (data not shown), this explanation is not sufficient. Additionally, lycopene may possess certain characteristics that make it less suitable as a biomarker for dietary intake, including a relatively low responsiveness of plasma lycopene concentrations to dietary intake (24).

Correlations between biomarkers and fruit and vegetable juice were low (data not shown). Vitamin C and carotenoid content in juice might be lower than that in fresh fruit. Alternatively, participants might have wrongly considered certain soft drinks to be fruit juices.

The findings of the present study must be interpreted with caution, because all participants were nonsmoking women with children in the same age group, and only 5 percent had a low educational level. Since the requirements of the study were many, it is likely that most of the women were highly motivated to participate. Moreover, it turned out that participants who dropped out of the study before it ended consumed fewer vegetables than participants who fully completed the study. How this selection bias affected the results is unknown, but participants in the present study may have been more aware of their fruit and vegetable consumption and/or may have completed the FFQ more accurately than would have been the case in a more representative population; this could have led to higher correlations between FFQ estimates and biomarkers. Generalization of the findings is further limited by the fact that all measurements in the present study were done in the spring. This minimized seasonal influences on biomarkers and fruit and vegetable consumption (and therefore increased the internal validity of our study), but we cannot be sure that the validity and reproducibility of the FFQ in other seasons would be similar to that presented here.

We conclude that the correlations with biomarkers we found are comparable to most correlations found in the studies mentioned above. Thus, our questionnaire appears suitable for ranking individuals according to their fruit and vegetable intake. Our FFQ also seems quite well able to rank persons according to changes in fruit and vegetable intake, but since data from the literature on associations of changes in fruit and vegetable intake with biomarkers are scarce, this is a preliminary conclusion. The reproducibility of the FFQ would appear to be satisfactory, though caution must be taken with regard to its long-term reproducibility for fruit consumption. Since this FFQ is short, cheap, and easy to implement, it can be a valuable dietary assessment instrument for epidemiologic studies and for evaluating the effectiveness of fruit and vegetable promotion interventions. However, the relative validity of the FFQ presented here will have to be extended to males, other age groups, and populations of lower educational levels.

ACKNOWLEDGMENTS

This research was supported by the Dutch Product Board for Horticulture.

REFERENCES

1. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. J Am Diet Assoc 1996;96:1027–39.
2. Gandini S, Merzenich H, Robertson C, et al. Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. Eur J Cancer 2000;36:636–46.
3. Voorrips LE, Goldbohm RA, Verhoeven DT, et al. Vegetable and fruit consumption and lung cancer risk in the Netherlands Cohort Study on diet and cancer. Cancer Causes Control 2000; 11:101–15.
4. Slattery ML, Benson J, Curtin K, et al. Carotenoids and colon cancer. Am J Clin Nutr 2000;71:575–82.
5. Bazzano LA, He J, Ogden LG, et al. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am J Clin Nutr 2002;76:93–9.
6. Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. Int J Epidemiol 1997;26:1–13.
7. Law MR, Morris JK. By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease? Eur J Clin Nutr 1998;52:549–56.
8. Liu S, Manson JE, Lee IM, et al. Fruit and vegetable intake and risk of cardiovascular disease: The Women's Health Study. Am J Clin Nutr 2000;72:922–8.
9. Netherlands Bureau for Food and Nutrition. Practical foodstuff guide. (In Dutch). The Hague, the Netherlands: Voedingscentrum, 1992.
10. Commissie Richtlijnen Goede Voeding. Guidelines for a healthy diet: recommendations drawn up by the Committee on Guidelines for a Healthy Diet. (In Dutch). The Hague, the Netherlands: Dutch Nutrition Council, 1986.
11. Benito E. Overview of dietary recommendations on diet and cancer. In: Benito E, Giacosa A, Hill MJ, eds. Public education on diet and cancer. Dordrecht, the Netherlands: Kluwer Academic Publishers, 1992:3–12.
12. Naska A, Vasdekis VG, Trichopoulou A, et al. Fruit and vegetable availability among ten European countries: how does it compare with the 'five-a-day' recommendation? DAFNE I and II projects of the European Commission. Br J Nutr 2000;84: 549–56.
13. Ammerman AS, Lindquist CH, Lohr KN, et al. The efficacy of behavioral interventions to modify dietary fat and fruit and vegetable intake: a review of the evidence. Prev Med 2002;35:25–41.
14. Kristal AR, Beresford SA, Lazovich D. Assessing change in diet-intervention research. Am J Clin Nutr 1994;59(suppl): 185s–9s.
15. Willett W, Lenart E. Reproducibility and validity of food frequency questionnaires. In: Willett W, ed. Nutritional epidemiology. New York, NY: Oxford University Press, 1998:101–47.
16. van 't Veer P, Kardinaal AF, Bausch Goldbohm RA, et al. Biomarkers for validation. Eur J Clin Nutr 1993;47(suppl 2): S58–63.
17. Tucker KL, Chen H, Vogel S, et al. Carotenoid intakes, assessed by dietary questionnaire, are associated with plasma carotenoid concentrations in an elderly population. J Nutr 1999; 129:438–45.
18. Campbell DR, Gross MD, Martini MC, et al. Plasma carotenoids as biomarkers of vegetable and fruit intake. Cancer Epidemiol Biomarkers Prev 1994;3:493–500.
19. Drewnowski A, Rock CL, Henderson SA, et al. Serum beta-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. Am J Clin Nutr 1997;65:1796–802.
20. van Kappel AL, Steghens JP, Zeleniuch Jacquotte A, et al. Serum carotenoids as biomarkers of fruit and vegetable consumption in the New York Women's Health Study. Public Health Nutr 2001;4:829–35.
21. Block G, Norkus E, Hudes M, et al. Which plasma antioxidants are most related to fruit and vegetable consumption? Am J Epidemiol 2001;154:1113–18.
22. John JH, Ziebland S, Yudkin P, et al. Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. Lancet 2002;359: 1969–74.
23. Zino S, Skeaff M, Williams S, et al. Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. BMJ 1997;314:1787–91.
24. Le Marchand L, Hankin JH, Carter FS, et al. A pilot study on the use of plasma carotenoids and ascorbic acid as markers of compliance to a high fruit and vegetable dietary intervention. Cancer Epidemiol Biomarkers Prev 1994;3:245–51.
25. McEligot AJ, Rock CL, Flatt SW, et al. Plasma carotenoids are biomarkers of long-term high vegetable intake in women with breast cancer. J Nutr 1999;129:2258–63.
26. Maskarinec G, Chan CL, Meng LX, et al. Exploring the feasibility and effects of a high-fruit and -vegetable diet in healthy women. Cancer Epidemiol Biomarkers Prev 1999;8:919–24.
27. Broekmans WM, Klopping Ketelaars IA, Schuurman CR, et al. Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. J Nutr 2000;130:1578–83.
28. Baranowski T, Smith M, Baranowski J, et al. Low validity of a seven-item fruit and vegetable food frequency questionnaire among third-grade students. J Am Diet Assoc 1997;97:66–8.
29. Block G, Gillespie C, Rosenbaum EH, et al. A rapid food screener to assess fat and fruit and vegetable intake. Am J Prev Med 2000;18:284–8.
30. Field AE, Colditz GA, Fox MK, et al. Comparison of 4 questionnaires for assessment of fruit and vegetable intake. Am J Public Health 1998;88:1216–18.
31. Serdula M, Coates R, Byers T, et al. Evaluation of a brief telephone questionnaire to estimate fruit and vegetable consumption in diverse study populations. Epidemiology 1993;4:455–63.
32. Thompson FE, Kipnis V, Subar AF, et al. Evaluation of 2 brief instruments and a food frequency questionnaire to estimate daily number of servings of fruits and vegetables. Am J Clin

Nutr 2000;71:1503–10.
33. Resnicow K, Odom E, Wang T, et al. Validation of three food frequency questionnaires and 24-hour recalls with serum carotenoid levels in a sample of African-American adults. Am J Epidemiol 2000;152:1072–80.
34. Plesko M, Cotugna N, Aljadir L. Usefulness of a brief fruit and vegetable FFQ in a college population. Am J Health Behav 2000;24:201–8.
35. Van Assema P, Brug J, Ronda G, et al. A short Dutch questionnaire to measure fruit and vegetable intake: relative validity among adults and adolescents. Nutr Health 2002;16:85–106.
36. Hulshof KF. The consumption of food groups by Dutch population groups: food consumption survey 1997–1998. (In Dutch). Zeist, the Netherlands: TNO Nutrition and Food Research, 1998.
37. Kistemaker C, Bouman M, Hulshof KF. The consumption of separate products by Dutch population groups: food consumption survey 1997–1998. (In Dutch). Zeist, the Netherlands: TNO Nutrition and Food Research, 1998.
38. Speek AJ, Schrijver J, Schreurs WH. Fluorometric determination of total vitamin C in whole blood by high-performance liquid chromatography with pre-column derivatization. J Chromatogr 1984;305:53–60.
39. Hess D, Keller HE, Oberlin B, et al. Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. Int J Vitam Nutr Res 1991;61:232–8.
40. Oostenbrug GS, Mensink RP, Hardeman MR, et al. Exercise performance, red blood cell deformability, and lipid peroxidation: effects of fish oil and vitamin E. J Appl Physiol 1997;83:746–52.
41. Bogers RP, Dagnelie PC, Westerterp KR, et al. Using a correction factor to correct for overreporting in a food frequency questionnaire does not improve biomarker-assessed validity of estimates for fruit and vegetable consumption. J Nutr 2003;133:1213–19.
42. Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 2003;133(suppl 3):933S–40S.
43. Forman MR, Lanza E, Yong LC, et al. The correlation between two dietary assessments of carotenoid intake and plasma carotenoid concentrations: application of a carotenoid food-composition database. Am J Clin Nutr 1993;58:519–24.
44. Martini MC, Campbell DR, Gross MD, et al. Plasma carotenoids as biomarkers of vegetable intake: the University of Minnesota Cancer Prevention Research Unit Feeding Studies. Cancer Epidemiol Biomarkers Prev 1995;4:491–6.
45. Kardinaal AF, van 't Veer P, Brants HA, et al. Relations between antioxidant vitamins in adipose tissue, plasma, and diet. Am J Epidemiol 1995;141:440–50.
46. Kim DJ, Holowaty EJ. Brief, validated survey instruments for the measurement of fruit and vegetable intakes in adults: a review. Prev Med 2003;36:440–7.
47. Byers T, Trieber F, Gunter E, et al. The accuracy of parental reports of their children's intake of fruits and vegetables: validation of a food frequency questionnaire with serum levels of carotenoids and vitamins C, A, and E. Epidemiology 1993;4:350–5.
48. Michaud DS, Giovannucci EL, Ascherio A, et al. Associations of plasma carotenoid concentrations and dietary intake of specific carotenoids in samples of two prospective cohort studies using a new carotenoid database. Cancer Epidemiol Biomarkers Prev 1998;7:283–90.
49. Osler M, Heitmann BL. The validity of a short food frequency questionnaire and its ability to measure changes in food intake: a longitudinal study. Int J Epidemiol 1996;25:1023–9.
50. Coates RJ, Eley JW, Block G, et al. An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low-income black women. Am J Epidemiol 1991;134:658–71.